Study of Functional food properties using *Prosopis cineraria (L) Druce* ripened fruit pod's synbiotic efficacy and gum exudate hydrocolloidal systems in Indian traditional food models.

A
Research Proposal
(For partial fulfilment of Doctor of Philosophy degree)

MODY UNIVERSITY
OF SCIENCE AND TECHNOLOGY
LAKSHMANGARH • RAJASTHAN
A LEADING WOMEN'S UNIVERSITY

Supervisor
Prof. R.K Gaur

Submitted by
Poornima Saraswat
(Enrol. No. 090141)

Department of Biosciences
College of Arts, Science and Humanities
Mody University of Science and Technology,
Lakshmangarh(Sikar), Rajasthan-332311

Table of Contents

Table of Contents 2
Abstract 3
Introduction 4
Purpose of research 4
Objectives 6
Literature review 7
Aim and Hypothesis 7
Methods 12
Work plan 14
Expected deliverables and Significance 15
References 16

Student Signature _____

Supervisor Signature _____

Date 4th Dec' 2017

Abstract

Prosopis cineraria(L) druce is a leguminous tree from arid and semi-arid regions of the Thar Desert, major part of which lies in the western India. This species provides all five essentials; Forest, Fuel, Fodder, Fibre and Food. In the Indian medical system of 'Ayurveda' this tree is widely recommended for treatment of a variety of ailments. The plant's parts have been shown to contain numerous bioactive compounds. The gum from *P. cineraria* is also prescribed in applications where it can be used as cryopreservant and has cardioprotectant potential. The leaves and fruit pods are a rich source of antioxidants and contain 19% crude protein, 26% crude fibre and 56% carbohydrates. Hence, they can be a good protein source in the diet. Subsequently, the nutritional benefits associated with microflora management approaches, foods are the main vehicle for pro-, pre- and synbiotics. Also, linking the traditional fermented food models with raw materials from native wild plant species will add a totally new angle. It will be more understandable to those who need them the most i.e rural population living in harsh climatic zones of India. One of the traditional North Indian fermented drink made from pearl millet called 'Rabari' is regularly consumed specially in rural areas where the summer temperature is generally quite high. The fermentation from homemade buttermilk gives pearl millet better starch digestibility.

Addition of protein rich ripened pod's flour of *P.cineraria* might help in improving the protein digestibility of the above traditional drink, fruit syrup as well as in biscuits. The synbiotic efficacy of the *P.cineraria's* functional food model system derived from these model foods with added hydrocolloidal properties of its gum exudate will be an innovative and interesting study for better starch as well as protein digestibility facilitating better absorption of nutrients.

Introduction

Purpose of Research:

P. cineraria is the only tree which grows in the stressed and severe drought conditions of the arid regions in India. Its crude protein contents of seed oil, leaves and pods are 16.5%, 11.9% and 18% (RANI et al., 2013).

As the protein constitutes very important component of human diet and sufficient dietary energy (caloric) intake defines the nutrition. This plant's pods provides both i.e. sufficient daily protein requirements and daily calorie energy requirements (Gehlot, Bohra, & Purohit, 2008).

Health benefits from various components of the plant are enormous in preventing wide range of illnesses including protein and mineral deficiencies as pods are consumed as human food and other parts are used as traditional medicines for people in India. The pods are known to contain high micronutrients such as calcium, magnesium, zinc and iron (M. C. Gupta, Gandhi, & Tandon, 1974).

The research needs to include an investigation of the functional properties of *P. cineraria* tree species which may help to fulfil the nutritional needs of susceptible populations. Because of the nutritional benefits associated with microflora management approaches, foods are the main vehicle for pro-, pre- and synbiotics. Evaluation of the natural presence of prebiotics in traditional Indian fermented plant-based foods or addition to fermented milk products as a way of augmenting ''probiotic'' bacteria already naturally resident in the human intestine will be addition to new endeavours (Anukam & Reid, 2009).

Also, linking the traditional fermented food models with raw materials from native wild plant species will add a totally new angle which will be more understandable to those who need them the most i.e rural population living in harsh climatic zones of India. A traditional North Indian fermented drink made from pearl millet called 'Rabari' is regularly consumed specially in rural areas where the summer temperature is generally quite high. The fermentation from homemade buttermilk gives pearl millet better starch digestibility.

Addition of protein rich ripened pods of *P.cineraria* might help in improving the protein digestibility of the traditional drink. The use of starter culture for fermentation will be isolated from camel's milk because of the two reasons: 1) local availability in Thar Desert. 2) novel bacteriocins were reported from Lactobacillus strain in fermented camel milk (Lü et al, 2014).

General description:

In Ayurveda, the Indian medical system, khejri known by synonym 'Shami' is widely prescribed for the treatment of a variety of ailments.

Scientifically known as *P. cineraria*, a native to tropical Indian desert and grows wildly with adaptation to hot and dry climate of the desert. It, therefore, provides support to the folklore in such a harsh environment to survive.

The present traditional food uses of *P. cineraria* do not have strong evidences of potential health benefits and clinical research. The fundamental research conducted so far on the plant gives only a brief idea for quotes that this plant has a tremendous potential for the development of functional foods with therapeutic values.

Hence, the research could be undertaken to study and innovations with inheriting high protein concentration in functional foods. Pods and gum exudates of the plant could be included into the

functional food model system and tested for its inheritance in high protein content, fiber index, glycemic response and In- vitro digestibility.

First reporting of the study of food value for unripe pods of *P. cineraria* was given by (M. C. Gupta et al., 1974). They found this study to be worthwhile because of the survey they were conducting in Mahendragarh district of Haryana state in India. In this survey, they found that with semi-desert conditions there was total absence of severe grades of protein-calorie malnutrition in the community due to consumption of substantial quantity of unconventional food in the form of P. cineraria pods. They reported that the whole unripe pods (sangri) contained protein, 15.8; fat, 3.0; and carbohydrate, 50.8 g/100gm dry weight.

The phytochemical and antioxidant assay were qualitatively investigated on unripe pods commonly known as sangri. Its moisture content was reported low (8.55±0.341%) which may be advantageous in better shelf life, carbohydrate content was 51.01±1.179% while protein came out to be 28.42±0.56% followed by fat content of 2.30±0.328%. Mineral content by ICP-OES analysis showed the presence of P, Mg, Zn, Se, Ca and Fe in good quantity. Moderate quantity of alkaloids and saponins were reported while tannin content was very high (Malik Shruti, 2013).

The present traditional food uses of *P. cineraria* do not have strong evidences of potential health benefits and clinical research. The potential for food production from *Prosopis* genus in truly arid or semi-arid regions where *Prosopis* has become widely naturalized, has not been touched (Felker, 2009). Its species *P. cineraria* has a true importance among the people as a food and fodder source in the Indian desert State. Its high-sugar pod flour served ~ 100 gm/day can provide an average of 340 calorie energy requirements daily.

The outcome of this project will be a validated functional food under most acceptable food model system and inheriting high protein content to combat Protein-calorie malnutrition which will help the local tribes not only in food with improved nutritional quality but also economic support for small scale industry depending on it.

Objectives of the proposed research

Major societal challenges are to combat nutritional imbalance, maintain sustainable food security and economic betterment. The Indian scenario of its semi-arid and arid zone vegetation indicates that it comprises of a wide variety of edible fruit-bearing plant species and *P. cineraria* is among one of them. They are undervalued and underutilized as more and varied exotic fruits are now easily accessible. With the growing modern global nutrition related problems, time has come to enhance and strengthen traditional food systems which are answer to them as 'complementary medicines'. Hence, *P.cineraria's* pods as well as gum exudates are proposed to put together in form of a functional food with synbiotic efficacy which can answer the modern world problems targeted as: Protein-calorie malnutrition.

Target steps for achieving the above-mentioned objectives are as follows:

- Collection of *P. cineraria* samples from three different districts viz. Sikar, Churu and Jhunjhunu of the Shekhawati region in Rajasthan.
- To develop the pure starter culture from camel's milk for fermentation and to be used in one of the functional food model system for probiotic efficacy.
- To develop functional food model system w.r.t Traditional recipies of
  a. 'fermented pearl millet drink'
  b. 'Fruit syrup'- using pods' flour and gum exudates of *P.cineraria* for assessment in hydrocolloidal systems
  c. 'Biscuits' for inheriting high protein content.
- To check the total fiber index, glycemic response and in-vitro digestibility of protein and starch content of the above developed food models.

Literature review

The potential for food production from *Prosopis* genus in truly arid or semi-arid regions where *Prosopis* has become widely naturalized, has not been touch (Felker, 2009). Its species *P. cineraria* has a true importance among the people as a food and fodder source in the Indian desert State. Its high-sugar pod flour served ~ 100 gm/day can provide an average of 340 calorie energy requirements daily. However, qualitatively least is known towards the application involved with gum exudate and the ripened pods.

The first generation of functional foods involved supplementation of constituents like calcium or vitamins, for their recognised health attributes. In more recent years, the concept has become more directed towards food additives that may exert a positive effect on the gut microbiota composition. This has largely concentrated on probiotics, but more recently interest in prebiotics has increased (Ziemer & Gibson, 1998).

Because of the nutritional benefits associated with microflora management approaches, foods are the main vehicle for pro-, pre- and synbiotics. Evaluation of the natural presence of prebiotics in traditional Indian fermented plant-based foods or addition to fermented milk products as a way of augmenting ''probiotic'' bacteria already naturally resident in the human intestine will be addition to new endeavours (Anukam & Reid, 2009).

Wild plants were originally the main element in the human diet, culminating in the diverse cultures and societies of today. However, the establishment of agriculture led to the decline of consumption of wild plants in comparison to the cultivars that could be grown every year (Grivetti & Ogle, 2000). Nevertheless, consumption of wild plants is still a tradition that remains in many cultures, either for their nutritional and health benefits or for sociocultural behaviours that characterize many societies (De Groot, Wilson, & Boumans, 2002); (Pardo-de-Santayana et al., 2007); (Schulp, Thuiller, & Verburg, 2014). As human health and nutrition are two of the pillars that sustain our survival, it is necessary to find innovative ways to support medical care, which can be found in the vast wild plant ecosystem (Heywood, 2011).

Functional foods, nutraceuticals, and drugs based on wild plants that are still unexplored are emerging as a response to the world market, which has been searching for new, better and safer products (Pinela et al., 2017).

Functional foods have a similar appearance to their traditional counterparts, but bring potential beneficial effects when consumed on a regular basis in a varied diet (Gulati & Ottaway, 2006).

National status:

P.cineraria-

Prosopis is a group of trees that stands out as having immense potential to help feed local folks and their livestock, especially the locally grown tree species of *Prosopis cineraria*. This is one of the most numerous and widespread tree in arid and semi-arid areas of Thar Desert. Although, some scientific studies have been undertaken on all of its parts with tremendous rise in interest for the last five years on the medical and food/fodder application for this tree, it still remains one of the most underutilized plant species (Girase, Jadhav, & Jain, 2016).

The extract of *P.cineraria* bark has been pharmacologically tested for gastrointestinal, respiratory and vascular diseases (Janbaz et al., 2012), anticonvulsant activity (Velmurugan, Arunachalam, &

Ravichandran, 2012), hematological activity (Purohit & Ram, 2012a) and anti-venom activity against *Naja naja* crude venom (Sivaraman, Rajesh, & Elango, 2013), hepatoprotective activity (Velmurugan & Arunachalam, 2014), hypolipidemic and antiatherosclerotic effects also analysed (Purohit & Ram, 2012b), nootropic activity (Bithu, Reddy, Prasad, Sairam, & Hemalatha, 2012).

Physico-chemical activities from chemical analyses of bark have been analysed and two new compounds isolated (Soni, Basak, Parasher, & Dobhal, 2015). Properties of the wood carbon in aqueous solution were investigated for waste water treatment such as adsorption of water soluble dyes (Manickam, 2016), removal of rhodamine 6G (Murugan, Jansi Rani, Subramaniam, & Subramanian, 2014) and adsorption potentialities of bio-sorbents for methyl orange dyes (Karimulla & Ravindhranath, 2013). The bark and leaf extracts were tested for their cytotoxicity and it was observed that there is marked increase in the number of MCF-7 and HeLa cells with the typical features of apoptosis, consisting in cells with condensed and fragmented nuclei. This study has proved the traditional claim for the plant in the management of cancer (Robertson, Narayanan, & Ravichandran, 2015).

The leaves of *P.cineraria* were studied for its Phytochemicals and pharmacognostic studies (sumitra singh, 2013). Its powder is studied in green remediation for removal of Chromium (VI) and Manganese (VII) (Sinha, Bhati, Sumit, & Gupta, 2013); (Virvadiya, Kumari, Choudhary, & Gupta, 2014), wound healing property (Abhishek Gupta, Verma, Gupta, Jangra, & Pratap, 2015), immunosuppressive and immune-pharmacological activity of flavonoid and saponin isolated from leaf extract (A Gupta & Chaphalkar, 2015a, 2015b), antimalarial activity (Satish, Somaiah, Brahmam, Rekha, & Sunita, 2015), anti-leprotic activity (Solanki, 2016) and anti-hyperlipidemic potential was also analysed against hyperlipidemia induced lab rats (D. Sharma & Singla, 2013b). The crude extract of leaves was tested for reproduction indexes in normal albino female mice (Rajvanshi & Garg, 2015), antioxidant activity (Dharani, Sumathi, Sivaprabha, & Padma, 2011) and antibacterial activity specially against *Staphylococcus* and *E.coli* (S. Sharma, Singh, Maherchandani, & Kashyap, 2012). Gallic acid and 4-hydroxy benzoic acid were isolated from leaf extract (D. Sharma & Singla, 2013a) and on a percentage dry-matter basis, leaves contain around 15.5 % crude protein and 7% tannins (Dutta, Sharma, & Hasan, 1999)

The fruit of *P.cineraria* is highly nutritious and most edible part of the plant. Unripe pods called 'sangri' are consumed as vegetable by local people while ripe pods called 'khokha', get dried and fall on ground at maturation. They are sweet in taste and eaten by people as well as livestock. The proximate analysis for the pods on percentage dry matter basis has shown 28% protein, 51% carbohydrate, 3% fat and rich in fiber (Malik Shruti, 2013). Physiochemical examination of fruits showed presence of alkaloids, saponins, tannins, flavonoids, cardiac glycosides and anthraquinones (Liu, Singh, & Nair, 2012).

The process to make raw pods suitable for vegetable preparation includes blanching and dehydration process. Hence, (Nagaraja, Saroj, Singh, & Awasthi, 2014) explained the effect on quality of pods after rehydration. (Malik Shruti, 2013) writes about the potential aspect of pods as functional food.

Traditional Functional foods w.r.t Northwestern India:

Traditional fermented food consumed regularly by people in the northwestern states of India. It is prepared by mixing pearl millet whole flour with country buttermilk and then fermenting the mixture for 4-6 hr at 35-45°C (Dhanker Neerja, 1987). It is called 'Rabadi' in common language.

Pearl millet (*Pennisetum typhoides Rich.*) constitutes a major source of protein and calories in the diet of millions of the Indian population. Protein digestibility of pearl millet like that of other plant

foods is low (Khetarpaul & Chauhan, 1990). Starch, a major component of carbohydrates in pearl millet, is relatively more resistant to attack by pancreatic amylase. Fermentation has been known to improve protein digestibility as well as starch digestibility of food grains (Dhanker Neerja, 1987).
Dhanker *et al* studied the effect of temperature and period of fermentation on protein and starch digestibility (*In-vitro*) of Rabadi- A Pearl Millet Fermented Food and found that Maximum increase in the digestibility of both protein (51%) and starch (58%) occurred after 9 hr fermentation at 45°C (Dhanker Neerja, 1987).
Khetarpaul et al studied Fermentation of pearl millet flour with yeasts and lactobacilli: *in vitro* digestibility and utilisation of fermented flour for weaning mixtures and found single as well as mixed culture fermentations by yeasts (S. diastaticus; S. cerevistiae) and lactobacilli (L. brevis; L. fermentum) at 30 °C for 72 h improved the starch and protein digestibility (in vitro) of pearl millet flour significantly (Khetarpaul & Chauhan, 1990)
Elnour et al experimental data indicated that in rats the enhanced effects of millet on the thyroid induced by fermentation is likely related to removal of minerals from millet and/or chemical transformation of the goitrogens contained in millet (Elnour et al., 1998).
Modha et al contributed the study for optimization of fermented milk beverage using pearl millet where the product developed using 5.3% flour and 72% water on the basis of curd gave the most acceptable product. For further stabilization during storage, pectin and/or carboxy methyl cellulose were tried at various levels and a level of 0.6% pectin was selected. The standardized product in glass bottles when stored under refrigeration (5–7 °C) showed the shelf life of 7 days (Modha & Pal, 2011).
Basu et al aimed the study to develop pearl millet based fermented skim milk product. The strain, *L. rhamnosus* RSI3 isolated from indigenous cereal based fermented milk product Rabaadi was screened and finally selected for starter formulation based on appreciable phytate degrading activity and significant antibacterial and antifungal activity against pathogens paired with a prolific acid producing compatible *S. thermophilus* ST20 (Basu & Tomar, 2016).

Starter culture of LAB (Lactic Acid Bacteria) from Camel's milk:
Nanda et al reported thirty-two Lactobacilli strains were isolated from four samples of camel cheese collected from Bikaner, India. These isolates were identified based on phenotypic and genotypic characteristics. Sequencing of 16S rDNA was performed for species identification and diversity analysis. *Lactobacillus delbrueckii* and *Lb. fermentum* were found to be dominant species followed by *Lb. plantarum* and *Lb. casei*. On evaluation of technological properties of these isolates, 20 isolates were observed to be good acid producers (Nanda et al., 2011).
(A. Patel, Patel, Patel, & Chaudhary, 2016) reviewed the importance of camel's milk. Indian camels yield 3.5 kg to 10 kg of milk per day. The taste of camel milk depends upon availability of plants for grazing. Unlike cow milk, it was found that camel milk can be preserved for a longer time at 30° C and most importantly the camel milk can be kept at 4° C for more than three months without any visible change. The ratio of fat to total solids average 31.6 percent. This is much lower than that of the buffalo, which is 40.9 percent. Camel milk is rich in vitamin C.

International status:
P.cineraria is the key-stone tree species of the Thar Desert which is present not only in the major part of Rajasthan on the Indian side but extends up to Sindh area in Pakistan. There is only scanty

research work carried out on this particular plant species which includes physiochemical characterization and comparison of its gum exudate with that of *P.Glandulosa* (Moghal, 2014), new antibacterial compounds isolation from aerial parts (Neghabi-Hajiagha et al., 2016), anti-pyretic investigations on leaves and fruit extracts (Ahmad, Qasier Jabeen, & Khan, 2013) and study on in-vitro gas production kinetics of leaves (Elahi et al., 2014).

However, a good amount of research is being carried out involving other plant species of the genus *Prosopis* of which the khejri tree species is also a part. Various literature reviews have mentions for a vast potential on functional food uses from different species of this genus (S. Patel, 2015) (Felker, 2009). The work being carried on *P.pallida's* seed galactomannan characterization (Alcedo, 1999), various forms of functional food being prepared and studied on its nutritional aspects with other species of this genus such as *P.juliflora, P.glandulosa, P.pallida, P.chilensis, P.nigra and P.alba* in the forms of different pod processing (Felker, Takeoka, & Dao, 2013);(Bernardi.C, 2006):

In general, pod processing involves drying, pounding, grinding or milling of pods, either as a single process producing a whole pod extract or with some separation of pod parts for further processing of each fraction. For hand grinding or milling in particular, an adequate drying step is essential to reduce the problems caused by moist mesocarps sticking during processing (Felker & Moss, 1996).

Two traditional products obtained from *Prosopis* in Peru are mainly based on the soluble sugars of the pulp. Also, mesquite pods occupied a central role in the Sonoran desert in North America, where the Indian tribes cooked the green pods with meat. Today in Northern Argentina, flour made from the sugary pulp of *Prosopis* species is known as "patay" and is still consumed (Felker & Moss, 1996).

In addition to that, a syrup "algarrobina" is made from whole or crushed pods from. *P. pallida* after being soaked in water for two hours, pressing, filtering and finally concentrating the liquid by evaporation. The flour is converted into an instantly soluble powder and could be used as a cocoa powder substitute. Coffee substitute has been also made from *P. juliflora* by roasting the coarse pulp flour at 120° C until it becomes dark brown during which time it agglomerates into large granules requiring further grinding. The final product is used in the same way as filter coffee granules. Furthermore, decent quality galactomannan gums for use in food and pharmaceutical industries could be made from *P. pallida* seeds but the process is complicated.

Also, the flour from mesquite pods can add a sweet, nutty taste to breads or used to make jelly or wine. When flour is used in baking, the flour is used in combination with other flour's substitute (1/4cup or 1/2cup). Mesquite flour is also used in pancakes, muffins, cakes and even cookies. Pods from *P. juliflora and P. pallida* complex could be used to supplement human diets (Fisher, 1977);(Simpson, 1977).

Similar traditional functional foods w.r.t Foreign countries:

Elnour et al stated that epidemiological evidence indicated that millet might play a role in the etiology of endemic goiter. They claimed that a traditional fermentation procedure of two pearl millet (*Pennisetum americanum L. Lecke*) cultivars grown in Sudan modified their effects on the weight of the thyroid gland and thyroid hormone profile in rats. They also reported that this fermentation procedure reduced the ash contents of millet by about 40% and removed considerable amounts of Mg (150%), Zn (27–39%) and K (45%). Other minerals (Ca, Fe, Cu) were not affected (Elnour et al., 1998).

In tropical area, cereal grains are milled and used to produce thick porridges, which are known by various names in different parts of the continent. In West Africa, particularly in Nigeria, Ghana and

Burkina Faso, one of such thick porridge is called 'Fura'- a semi-solid dumpling cereal (Anukam & Reid, 2009).

Inyang et al investigated on effect of germination and fermentation of Pearl millet on proximate chemical and sensory properties of instant 'Fura'- A Nigerian Cereal Food. Standard assay. Standard assay procedures were used to evaluate the fura samples for nutrient composition and phytic levels. Germination and fermentation increased protein, ash, crude fibre, phosphorous, Calcium and Iron levels of fura samples. (C.U Inyang, 2008)

Starter culture of LAB (Lactic Acid Bacteria) from Camel's milk:

Camel milk contained lactic acid producing bacteria like Streptococci such as *S. cremoris* and *S. lactis* and Lactobacilli such as *L. acidophilus*. *L. acidophilus* grew more rapidly in camel milk than others as its growth was supported by camel milk. Ability of each strain was tested to convert lactose of milk into lactic acid. It was observed that 66% lactose was converted by *S. lactis* 20, whereas *S. cremoris* 22 and *L. acidophilus* 23 converted 56 and 74% lactose into lactic acid, respectively. This was reported by (T. Ahmed & Kanwal, 2004).

Fguiri et al aimed their work to study the suitability of camel milk to produce dairy products by lactic acid fermentation. Sixty strains of lactic acid bacteria (LAB) were isolated from camel milk. Two strains of *Lactococcus lactis* SCC133 and SLch14 were selected to produce traditional Tunisian fermented dairy products (Lben, Raib, Jben cheese and Smen). These strains were chosen based on their acid production capacity and their ability to produce a high yield of biomass (Fguiri et al., 2016).

Hawaz et al Characterized lactic acid bacteria from camel milk and their technological properties to use as a starter culture and concluded that *Lactobacillus plantarum* HUM19, *Lactobacillus acidophilus* HUM20, and *Streptococcus cremoris* HUM8 had high acidifying, antimicrobial and proteolytic activities, and EPS production among all other lactic acid bacteria isolates (Hawaz, Guesh, Kebede, & Menkir, 2016).

AIM

To develop a potential food product(s) that will be based on traditional food model system in which synbiotic efficacy and hydrocolloidal property of *P.cineraria's* pods flour and gum exudate's will be tested for better protein digestibility and its absorption.

Hypothesis

The functional food product(s) developed from *P.cineraria's* fruit pods and gum exudates will be holding better protein and starch digestibility (*In-vitro*) facilitating better absorption.

Methods

Collection of samples:

The first year of research work will begin with the collection of sample materials by scouting method from-

For plant species' samples will be collected from Rajasthan state in three batches:

Collection Batch I: Jhunjhunu District

Collection Batch II: Sikar District

Collection Batch III: Churu District

Camel milk will be procured from National Research Centre for Camel, Bikaner, Rajasthan.

Sample processing: Samples will be washed and processed accordingly and stored at -80°C for further processing.

Total proximate and physicochemical analysis:

The physiochemical and total proximate analysis is to be determined according to the standard methods of the Association of Official Analytical Chemists (AOAC, 1990).

Pure starter culture isolation from camel's milk:

Lactic acid bacteria (LAB)- *Lactobacillus plantarum*, *Lactobacillus acidophilus*, and *Streptococcus cremoris* will be isolated from the camel milk samples using de Man Ragosa and Sharpe (MRS) and M17 agar according to the method described by (Harrigan & McCance, 1976).

After obtaining pure culture, following biochemical tests will be performed for identification purposes (As per Bergey's manual):

1. Catalase test. 2. Gram's staining. 3. Motility. 4. Acidification of sugars (sugar tests).
5. Growth in 4.0 and 6.5% NaCl. 6. Growth on 0.3% methylene blue. 7. Temperature tolerance test.

Lactose estimation:

After isolation and identification of LAB, ability of each strain for conversion of lactose into lactic acid will be tested by the method of (A. Ahmed, Rao, & Ramanatham, 1988).

Ability of isolated LAB to convert lactose into lactic acid:

After adding the starter culture for cheese making in fresh camel milk in following concoction: *L.plantarum: S.cremoris* and *L.acidophilus: S.cremoris* cultures in 95:5 ratios each (T. Ahmed & Kanwal, 2004).

Total titratable acidity, expressed as % lactic acid, will be measured by the method of (Atherton & Newlander, 1977) and pH will be recorded.

Development of Functional food* model system:

*"functional foods". These are thought to provide benefits beyond basic nutrition and may play a role in reducing or minimizing the risk of certain diseases and other health conditions. By knowing which foods can provide specific health benefits, one can make food and beverage choices that allow them to take greater control of their health.

Formulation of Synergistic food model system.

It will be a combination of *P.cineraria* pods, gum and other ingredients tested for their high protein inheriting ability following particular procedure for standardization in recipes as follows:

a. **Fermented pearl millet drink-** With the isolated strains as starter cultures, method of development of cereal based fermented milk product to be standardized using defatted camel milk, ripened pod's flour, ungerminated pearl millet (*Pennisetum glaucum*) flour as raw material and cumin (*Cuminum cyminum*), black pepper (*Piper nigrum*), curry patta (*Murraya koenigii*) and salt as flavouring agents. *P.cineraria's* gum exudate will be used as hydrocolloidal agent and stabilizer.

b. **Fruit syrup-** Extract of grinded ripened pods of *P.cineraria* will be combined with different ratios of gum exudates, pectin, jaggery and sugar.

c. **Biscuit-** grinded ripened pods of P.cineraria will be combined with rice flour, ragi flour, bajra flour, cocoa powder, unsalted butter and sugar.

Overall comparison will be done with following methods:

1. **Proximate analysis (AOAC, 1990).** Protein, fat and ash contents will be estimated using standard methods of analysis. Soluble, insoluble and total dietary fibre content will be investigated by employing enzymatic method (AOAC, 1991). Total carbohydrate content will be measured by difference, subtracting 100g minus the sum of grams of water, proteins, lipids, ash and dietary fibre.
2. **Experimental processing methods:** for determination of optimum conditions that should be followed for production of an ideal food model system with various permutations and combinations of recipes.
3. ***In-vitro* Digestibility tests.**
   w.r.t Starch and Protein
   Study will be based on (Akeson & Stahmann, 1964) method.

Functional Properties evaluation:

1. **Determination of Antioxidant capacity.**
2. **Study of emulsifying activity and stability of gum.**

Work Plan

Timeline	**Work responsibilities**
1st Year	Collection of Indian plant *P.cineraria's* materials. Sample processing. Pre- Proximate and physiochemical analysis.
2nd Year	Development of pure starter culture: Isolation and Characterization. Development of food model
3rd Year	Food model: Comparision for proximate, digestibilities of protein and starch. Functional properties evaluation. Writing literature, scientific data and standardization.

Expected deliverables/outcome

A validated functional food of most acceptable food model system and inheriting high protein, fibre and natural sugars to combat Protein-energy malnutrition and nutrition imbalance which will help the nationals from both the countries not only in food with improved nutritional quality but also socio-economic support for small scale industry depending on it.
Major aspect of the following functional food will be its high digestibility which will in turn help in regulate the glycemic index and controlling the cholesterol levels defining the food as antidiabetic and capable in providing balance nutrition.

Significance of the expected outcome

The big idea: Stimulate health and wealth especially in rural areas by providing communities access to healthy locally fermented foods produced by dairy local cooperatives.

How?: Enabling the production of a functional fermented drink via training programs.

Benefits: Reduction of milk spoilage, improvement of digestibility, nutritional enrichment, added commercial value, health benefits by use as a delivery vehicle for a Synbiotic.

Synbiotics are not present where they can make a difference!
-Diseases and associated malnutrition remain a leading cause of mortality and morbidity of children in India

References

Ahmad, M., Qasier Jabeen, M. W., & Khan, H. M. S. (2013). Time and dose dependent antipyretic investigations of ethanolic leaves and fruits extracts of Prosopis cineraria L.(Druce).

Ahmed, A., Rao, A. A., & Ramanatham, G. (1988). Effect of autofermentation on the physicochemical properties of proteins of sorghum-groundnut composite flour. *Journal of Agricultural and Food Chemistry, 36*(4), 690-694.

Ahmed, T., & Kanwal, R. (2004). Biochemical characteristics of lactic acid producing bacteria and preparation of camel milk cheese by using starter culture. *Pakistan Veterinary Journal, 24*(2), 87-91.

Akeson, W. R., & Stahmann, M. A. (1964). A pepsin pancreatin digest index of protein quality evaluation. *Journal of Nutrition, 83*, 257-261.

Alcedo, G. E. C. (1999). *Production and characterisation of Prosopis seed galactomannan.* Universidad de Piura.

Anukam, K. C., & Reid, G. (2009). African traditional fermented foods and probiotics. *Journal of medicinal food, 12*(6), 1177-1184.

AOAC. (1990). Association of Official Analytical Chemistry Official Methods of Analysis. *Assoc. of analytical chemistry, Washington D.C., U.S.A., 15*.

Atherton, H. V., & Newlander, J. A. (1977). *Chemistry and testing of dairy products*: AVI Publishing Co. Inc.

Basu, S., & Tomar, S. K. (2016). Development of novel indigenous pearl millet based fermented Skim milk product. *International Journal of Fermented Foods, 5*(1), 39.

Bernardi.C, D. S., Sabbag.N, Sanchez.H & Freyre.M. (2006). Formulation and sensory evaluation of Prosopis alba (algarrobo) pulp cookies with increased Iron and Calcium Dialyzabilities. *Plant Foods for Human Nutrition, 61*, 39-44.

Bithu, B. S., Reddy, N. R., Prasad, S. K., Sairam, K., & Hemalatha, S. (2012). Prosopis cineraria: A potential nootropic agent. *Pharmaceutical biology, 50*(10), 1241-1247.

C.U Inyang, U. M. Z. (2008). Effect of germination and fermentation of pearl millet on proximate, chemical and sensory property of instant 'Fura'- A Nigerian cereal food. *Pakistan Journal of Nutrition, 7*(1), 9-12.

De Groot, R. S., Wilson, M. A., & Boumans, R. M. (2002). A typology for the classification, description and valuation of ecosystem functions, goods and services. *Ecological economics, 41*(3), 393-408.

Dhanker Neerja, C. B. M. (1987). Effect of Temperature and Period of Fermentation on Protein and Starch Digestibility (In vitro) of Rabadi- A Pearl Millet Fermented Food *Journal of Food Science, 52*(2), 489-490.

Dharani, B., Sumathi, S., Sivaprabha, J., & Padma, P. (2011). In vitro antioxidant potential of Prosopis cineraria leaves. *J. Nat. Prod. Plant Resour, 1*(3), 26-32.

Dutta, N., Sharma, K., & Hasan, Q. (1999). Effect of supplementation of rice straw with Leucaena leucocephala and Prosopis cineraria leaves on nutrient utilization by goats. *Asian Australasian Journal of Animal Sciences, 12*, 742-746.

Elahi, M. Y., Nia, M. M., Salem, A. Z., Mansouri, H., Olivares-Pérez, J., Cerrillo-Soto, M. A., & Kholif, A. E. (2014). Effect of polyethylene glycol on in vitro gas production kinetics of Prosopis cineraria leaves at different growth stages. *Italian Journal of Animal Science, 13*(2).

Elnour, A., Liedén, S.-Å., Bourdoux, P., Eltom, M., Khalid, S., & Hambraeus, L. (1998). Traditional fermentation increases goitrogenic activity in pearl millet. *Annals of nutrition and metabolism, 42*(6), 341-349.

Felker, P. (2009). Unusual physiological properties of the arid adapted tree legume Prosopis and their applications in developing countries. *Perspectives in biophysical plant ecophysiology: A tribute to park S. Nobel*, 221-255.

Felker, P., & Moss, J. (1996). Prosopis: Semiarid fuelwood and forage tree building consensus for the disenfranchised. *Constitution, 13*, 15.

Felker, P., Takeoka, G., & Dao, L. (2013). Pod mesocarp flour of North and South American species of leguminous tree prosopis (mesquite): Composition and food applications. *Food Reviews International, 29*(1), 49-66.

Fguiri, I., Ziadi, M., Atigui, M., Ayeb, N., Arroum, S., Assadi, M., & Khorchani, T. (2016). Isolation and characterisation of lactic acid bacteria strains from raw camel milk for potential use in the production of fermented Tunisian dairy products. *International journal of dairy technology, 69*(1), 103-113.

Fisher, C. (1977). Mesquite and modern man in southwestern North America. *Mesquite: its biology in two desert ecosystems, 4*, 177-188.

Gehlot, P., Bohra, N., & Purohit, D. (2008). Endophytic mycoflora of inner bark of Prosopis cineraria-a key stone tree species of Indian desert. *Am Eur J Bot, 1*(1), 01-04.

Girase, M. V., Jadhav, M. L., & Jain, A. S. (2016). Prosopis Spicigera: A Nature's Gift. *International Journal of Pharmaceutical Chemistry and Analysis, 3*(1), 49-52.

Grivetti, L. E., & Ogle, B. M. (2000). Value of traditional foods in meeting macro-and micronutrient needs: the wild plant connection. *Nutrition Research Reviews, 13*(1), 31-46.

Gulati, O. P., & Ottaway, P. B. (2006). Legislation relating to nutraceuticals in the European Union with a particular focus on botanical-sourced products. *Toxicology, 221*(1), 75-87.

Gupta, A., & Chaphalkar, S. (2015a). Immunopharmacological Activity of Saponin from Terminalia arjuna and Prosopis spicigera. *J Pharma Reports, 1*(102), 2.

Gupta, A., & Chaphalkar, S. (2015b). Immunosuppressive activity of flavonoids isolated from Terminalia arjuna, Prosopis spicigera and Mimusops elengi. *vaccine, 16*, 1.98.

Gupta, A., Verma, S., Gupta, A. K., Jangra, M., & Pratap, R. (2015). Evaluation of Prosopis cineraria (l) druce leaves for wound healing activity in rats. *Annals of Pharma Research, 3*(01), 70-74.

Gupta, M. C., Gandhi, B., & Tandon, B. (1974). An unconventional legume-Prosopis cineraria. *The American journal of clinical nutrition, 27*(10), 1035-1036.

Harrigan, W. F., & McCance, M. E. (1976). *Laboratory methods in food and dairy microbiology*: Academic Press Inc.(London) Ltd.

Hawaz, E., Guesh, T., Kebede, A., & Menkir, S. (2016). Characterization of Lactic Acid Bacteria from Camel Milk and their Technological Properties to Use as a Starter Culture. *East African Journal of Science, 10*(1), 49-60.

Heywood, V. H. (2011). Ethnopharmacology, food production, nutrition and biodiversity conservation: towards a sustainable future for indigenous peoples. *Journal of ethnopharmacology, 137*(1), 1-15.

Janbaz, K. H., Haider, S., Imran, I., Zia-Ul-Haq, M., De Martino, L., & De Feo, V. (2012). Pharmacological evaluation of Prosopis cineraria (L.) Druce in gastrointestinal, respiratory, and vascular disorders. *Evidence-Based Complementary and Alternative Medicine, 2012*.

Karimulla, S., & Ravindhranath, K. (2013). Adsorption potentialites of bio-sorbents derived from prosopis cineraria and hibiscus rosa-sinensis in the removal of methyl orange dye from polluted waters.

Khetarpaul, N., & Chauhan, B. (1990). Fermentation of pearl millet flour with yeasts and lactobacilli: in vitro digestibility and utilisation of fermented flour for weaning mixtures. *Plant Foods for Human Nutrition (Formerly Qualitas Plantarum), 40*(3), 167-173.

Liu, Y., Singh, D., & Nair, M. G. (2012). Pods of Khejri (Prosopis cineraria) consumed as a vegetable showed functional food properties. *Journal of Functional Foods, 4*(1), 116-121.

Malik Shruti, M. S., Gupta Deepika, Gupta K Rajinder. (2013). Nutraceutical properties of Prosopis cineraria (L.) Druce Pods: A component of "Panchkuta". *Journal of Pharmacognosy and Phytochemistry, 2*(2), 66-73.

Manickam, J. R. (2016). Study of water soluble dyes adsorption from aqueous solution by Prosopis spicigera L. wood (PSLW) carbon. *Indian Journal of Chemical Technology (IJCT), 23*(1), 22-30.

Modha, H., & Pal, D. (2011). Optimization of Rabadi-like fermented milk beverage using pearl millet. *Journal of food science and technology, 48*(2), 190-196.

Moghal, M. (2014). Physicochemical Characterization of Gum Exuded From Prosopis Cineraria and Prosopis Glandulosa Species of Thar Desert Pakistan.

Murugan, M., Jansi Rani, M., Subramaniam, P., & Subramanian, E. (2014). Use of activated carbon prepared from Prosopis spicigera L. wood (PSLW) plant material for the removal of rhodamine 6G from aqueous solution. *Desalination and Water Treatment*, 1-11.

Naczk, M., & Shahidi, F. (2004). Extraction and analysis of phenolics in food. *Journal of Chromatography A, 1054*(1), 95-111.

Nagaraja, A., Saroj, P., Singh, I., & Awasthi, O. (2014). Effect of blanching treatments and dehydration methods on rehydration quality of khejri (Prosopis cineraria) pods. *The Indian Journal of Agricultural Sciences, 84*(9).

Nanda, D. K., Tomar, S. K., Singh, R., Mal, G., Singh, P., Arora, D. K., . . . Kumar, D. (2011). Phenotypic and genotypic characterisation of Lactobacilli isolated from camel cheese produced in India. *International journal of dairy technology, 64*(3), 437-443.

Neghabi-Hajiagha, M., Aliahmadi, A., Taheri, M. R., Ghassempour, A., Irajian, G., Rezadoost, H., & Feizabadi, M. M. (2016). A bioassay-guided fractionation scheme for characterization of new antibacterial compounds from Prosopis cineraria aerial parts. *Iranian journal of microbiology, 8*(1), 1.

Pardo-de-Santayana, M., Tardío, J., Blanco, E., Carvalho, A. M., Lastra, J. J., San Miguel, E., & Morales, R. (2007). Traditional knowledge of wild edible plants used in the northwest of the Iberian Peninsula (Spain and Portugal): a comparative study. *Journal of Ethnobiology and Ethnomedicine, 3*(1), 27.

Patel, A., Patel, S., Patel, N., & Chaudhary, G. (2016). Importance of camel milk-An alternative dairy food. *Journal of Livestock Science (ISSN online 2277-6214), 7*, 19-25.

Patel, S. (2015). Prosopis Genus as Food and Drug Repository: Exploring the Literature Databases *Emerging Bioresources with Nutraceutical and Pharmaceutical Prospects* (pp. 81-90): Springer.

Pinela, J., Carocho, M., Dias, M. I., Caleja, C., Barros, L., & Ferreira, I. C. (2017). Wild Plant-Based Functional Foods, Drugs, and Nutraceuticals. *Wild Plants, Mushrooms and Nuts: Functional Food Properties and Applications*, 315-351.

Purohit, A., & Ram, H. (2012a). Effect of Prosopis cineraria bark extract on hematology in hypercholesterolemic rabbits. *Indian J. Fund. Appl. Life Sci, 2*(2), 96-100.

Purohit, A., & Ram, H. (2012b). Hypolipidemic and antiatherosclerotic effects of Prosopis cineraria bark extract in experimentally induced hyperlipidemic rabbits. *Asian Journal of Pharmaceutical and Clinical Research, 5*(3), 106-109.

Rajvanshi, S., & Garg, V. (2015). Dose-specific response of aqueous crude extract of Prosopis cineraria on reproductive indexes in normal female Swiss albino mice. *International Journal of Research in Engineering and Applied Sciences, 5*(6), 139-145.

Rani, B., Singh, U., Sharma, R., Gupta, A. A., Dhawan, N. G., Sharma, A. K., . . . Maheshwari, R. K. (2013). Prosopis Cineraria (L) Druce: A desert tree to brace livelihood in Rajasthan. *Asian Journal of Pharmaceutical Research and Health Care, 5*(2).

Robertson, S., Narayanan, N., & Ravichandran, V. (2015). Investigation on the cytotoxic nature of leaf and stem bark extracts of Prosopis cineraria. *Asian Journal of Pharmaceutical and Clinical Research, 8*(2).

Satish, P., Somaiah, K., Brahmam, P., Rekha, N. S., & Sunita, K. (2015). Antimalarial activity of Prosopis cineraria (L) druce against chloroquine sensitive Plasmodium falciparum 3D7 strain.

Schulp, C., Thuiller, W., & Verburg, P. H. (2014). Wild food in Europe: A synthesis of knowledge and data of terrestrial wild food as an ecosystem service. *Ecological economics, 105*, 292-305.

Sharma, D., & Singla, Y. P. (2013a). Analysis of Gallic acid and 4-Hydroxy benzoic acid in Prosopsis cineraria leaf extract using High Performance Liquid Chromatography. *J Sci Innov Res, 2*, 790-794.

Sharma, D., & Singla, Y. P. (2013b). Evaluation of antihyperglycemic and antihyperlipidemic activity of Prosopis cineraria (Linn.) in wistar rats. *Journal of Scientific and Innovative Research, 2*(4), 751-758.

Sharma, S., Singh, J., Maherchandani, S., & Kashyap, S. (2012). Antibacterial activity of Ziziphus nummularia and Prosopis cineraria leaves extracts against staphylococcus aureus and Escherichia Coli. *Veterinary Practitioner, 13*(1), 46-48.

Simpson, B. B. (1977). Mesquite, its biology in two desert shrub ecosystems. *Mesquite, its biology in two desert shrub ecosystems.*

Sinha, R., Bhati, M., Sumit, M. L., & Gupta, V. (2013). Removal of Cr (VI) by Prosopis cineraria leaf powder-A green remediation. *Indian Journal of Chemical Technology, 20*(5), 312-316.

Sivaraman, T., Rajesh, S. S., & Elango, V. (2013). In vivo studies on detoxifying actions of aqueous bark extract of Prosopis cineraria against crude venom from Indian cobra (Naja naja). *Bangladesh Journal of Pharmacology, 8*(4), 395-400.

Solanki, R. (2016). In-Vitro Antileprotic Activity of Ethanolic Extracts of Mimosa Pudica L. and Prosopis Cineraria L.(Druce). *International Journal of Scientific Research, 4*(12).

Soni, L. K., Basak, A., Parasher, P., & Dobhal, M. P. (2015). Isolation and Structure Elucidation of Two New Compounds from Stem bark of Prosopis cineraria.

sumitra singh, v. n., S.K. sharma. (2013). Pharmacognistic studies on the leaves of Prosopis Cineraria (L) Druce growing in South Haryana, India. *Journal of Pharmacognosy and Phytochemistry, 2*(1), 320-325.

Velmurugan, V., & Arunachalam, G. (2014). Hepatoprotective activity of methanol extract of stem bark of Prosopis cineraria Linn against carbontetrachloride induced hepatotoxicity. *Int J Pharm Sci, 6*(suppl 2), 491-493.

Velmurugan, V., Arunachalam, G., & Ravichandran, V. (2012). Anticonvulsant activity of methanolic extract of Prosopis Cineraria (Linn) Druce stem barks. *Int J Pharm Tech Res, 4*(1), 89-92.

Virvadiya, C., Kumari, S., Choudhary, V., & Gupta, V. (2014). Combined bio and chemosorption of Mn (VII) from aqueous solution by Prosopis cineraria leaf powder. *European Chemical Bulletin, 3*(4), 315-318.

Ziemer, C. J., & Gibson, G. R. (1998). An overview of probiotics, prebiotics and synbiotics in the functional food concept: perspectives and future strategies. *International Dairy Journal, 8*(5-6), 473-479.